Cancer and Developmental Exposure to Endocrine Disruptors

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Developing organisms have increased susceptibility to cancer if they are exposed to environmental toxicants during rapid growth and differentiation. Human studies have demonstrated clear increases in cancer after prenatal exposure to ionizing radiation, and there is suggestive evidence that brain tumors and leukemia are associated with parental exposures to chemicals. Animal experiments have demonstrated increased tumor formation induced by prenatal or neonatal exposure to a variety of chemicals, including direct-acting carcinogens and drugs. Recently, natural estrogens have been classified as known human carcinogens. Prenatal exposure to natural and synthetic estrogens is associated with increases in breast and vaginal tumors in humans as well as uterine tumors in animals. Synthetic halogenated chemicals increase liver tumors after early life-stage exposure. Recently, a prototypical endocrine-disrupting compound, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, has been shown to be a developmental toxicant of the mammary gland in rodents. Dioxin alters multiple endocrine systems, and its effects on the developing breast involve delayed proliferation and differentiation of the mammary gland, as well as an elongation of the window of sensitivity to potential carcinogens. Implications of these new findings suggest that causes of endocrine-related cancers or susceptibility to cancer may be a result of developmental exposures rather than exposures existing at or near the time of tumor detection. *Key words:* animal models, atrazine, carcinogenesis, childhood cancers, development, dioxin, endocrine disruptors. *Environ Health Perspect* 111:389–394 (2003). doi:10.1289/ehp.5686 available via *http://dx.doi.org/* [Online 1 November 2002]

The developing child (fetus to prepuberty) is particularly susceptible to environmental insult, because development is a highly integrated process in which high rates of proliferation and extensive differentiation are coordinated with each other and with programmed cell death. Rapid growth rates allow for mutagenic and epigenetic alterations as cells proliferate. Likewise, differentiation represents a highly controlled process in which patterns of gene expression undergo massive changes. Thus, both cell division and differentiation offer multiple opportunities for the initiation of lesions as well as the promotion of the growth of altered cells; these are hallmarks of the complex process known as cancer. In addition, physiologic protective barriers such as the placenta and the blood–brain barrier are not complete *in utero*. Further, the metabolizing and elimination capabilities of the developing organism are not fully developed until after birth (Miller 1983), leaving the fetus and newborn particularly susceptible to adverse effects of environmental compounds.

Developmental Carcinogens in Humans

Early childhood exposures to infectious agents are key determinants for both hepatocellular carcinoma (Hsieh et al. 1992) and acute lymphoblastic leukemia (ALL; Greaves 1997). However, exposure to environmental compounds *in utero*—a proliferative period in human development—has long been thought to be critically involved in the causation of cancers in children and young adults. Although a wide range of potentially harmful agents may be involved, there is unequivocal evidence for two environmental agents: ionizing radiation and the estrogen agonist diethylstilbestrol (DES) (Anderson et al. 2000). The clear evidence for the role of ionizing radiation comes from studies involving diagnostic assessment of pregnant women. Doll and Wakeford (1997) have concluded that low doses of X ray to the fetus, especially during the last trimester, cause an increased risk of leukemia and all other types of cancer during childhood. In addition, therapeutic X ray of infants is also associated with thyroid and breast cancer later in life (Boice and Miller 1999). The clear association between fetal exposure to DES and vaginal adenocarcinoma in those young women (Herbst et al. 1971) led to the suggestion that developmental exposure to other hormonally active substances could also be associated with delayed cancer.

There is evidence for an association between prenatal exposure to a variety of environmental insults and childhood cancers. Both the timing and the chemical species may contribute to the actual risk of cancer to the child. Parental occupational exposure to various chemicals correlates with an increased incidence of brain tumors in their offspring (Peters et al. 1981). Shu et al. (1999) have recently shown that parental exposure to hydrocarbons is related to an increased risk of ALL in their children, and that preconceptional paternal exposure and postnatal maternal exposure to a variety of plastics was associated with an increased risk of childhood ALL. Maternal exposure to solvents, paints, or paint thinners from preconception to birth has a reported association with an increased risk of ALL. Freedman et al. (2001) recently reported an association between household organic solvent exposure and childhood ALL, including children whose mothers had painted extensively in the year before the child's birth. Parental use of pesticides has been associated with an increased risk of childhood leukemia or lymphoma (Meinert et al. 2000). Specifically, children living on farms may be at increased risk of leukemia, as the use of household pesticides, either by parents or professional applicators, was associated with childhood lymphoma. Alexander et al. (2001) recently demonstrated that *in utero* exposure to chemicals is associated with an elevated risk of acute leukemia in infants. The carbamate pesticide propoxur is one environmental chemical identified where exposure was highly correlated with leukemia in the offspring. Parental cigarette smoking has also been linked to childhood cancer (Pershagen 1989; Stjernfeldt et al. 1986). Even with several identified developmental triggers in the environment, it should be noted that because childhood cancers are so rare, it is very difficult to conduct prospective epidemiologic studies following toxicant exposures because large longitudinal studies would be required.

Early Life-Stage Carcinogens in Animal Models

The data from experimental animal studies for developmental exposures and early life-stage or adult cancer are far more extensive and convincing than the current epidemiologic data. Many of the animal data are well correlated

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We gratefully acknowledge the constructive comments on this review from D. Wolf, Office of Research and Development (ORD), NHEERL, Environmental Carcinogenesis Division; J. Cogliano, ORD, National Center for Environmental Assessment; and A. Mahfouz, Office of Water, Office of Science & Technology, at the U.S. EPA.

The research in this article has been reviewed by the National Health and Environmental Effects Research Laboratory, U.S. EPA, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Received 4 April 2002, accepted 13 September 2002.

with human data, whereas other data indicate compounds that deserve further study in human populations. A variety of types of radiation (e.g., X ray, ultraviolet) and chemicals given before mating, during pregnancy, or directly to the neonate can lead to an increased incidence of neoplasms in the offspring (Tomatis 1989). Preconceptional exposures during sperm or oocyte maturation have led to transgenerational carcinogenesis for several types of radiation and a variety of chemical carcinogens (reviewed in Tomatis 1989). For example, ethyl-nitrosourea (ENU) led to nervous system tumors in rats, and X ray caused lung tumors in mice. Urethane caused lung and liver tumors in mice, but the particular response was strain specific. Depending on periconceptional day, exposure to *N*-nitrosodiethylamine (NDEA) caused a variety of tumor types in hamsters. The actual effects of preconceptional exposure to these carcinogens seemed to vary with chemical, dose, and genetics. However, recent work by Yu et al. (1999) demonstrated that preconception exposure of the parental male mouse affected neoplastic changes in nearly all tissues in which tumors occur naturally with aging.

Anderson et al. (2000) have extensively reviewed the literature on carcinogenesis in offspring with environmental exposures to known carcinogens during gestation, and a summary of key findings is provided. In some cases the number of tumors (incidence or multiplicity) in exposed offspring were greatly increased after early life-stage exposure; in others, the time to spontaneous tumors (latency) was decreased by exposure. Because one would expect that direct carcinogens would consistently induce tumors in exposed offspring, the significance of the timing of developmental exposure must be reiterated. Skin carcinogenesis has been caused by prenatal X ray in mice. Transplacental initiation, followed by additional treatment with the chemical carcinogen dimethylbenz[*a*]anthracene (DMBA) after birth, also increased skin tumors in mice. Exposure of gravid hamsters with ENU led to melanomas in the offspring. Respiratory tumors have been observed in many mouse strains and in hamsters after prenatal exposure to a wide variety of direct-acting mutagens, including X ray, DMBA, urethane, ENU, and NDEA, among others. Likewise, ovarian and uterine tumors have been caused in mice by gestational exposure to X ray, DMBA, urethane, and ENU. In addition to lymphoid neoplasms in mice by X rays, urethane, DMBA, and ENU, among others, the same tumor type was caused by prenatal exposure of dogs to radiation Prenatal ENU led to nervous system tumors in mice, rats, hamsters, opossums, and rabbits, and to kidney tumors in mice, rats, hamsters, rabbits, and opossums. Prenatal X ray led to pituitary tumors in mice; DMBA caused forestomach tumors in mice, whereas ENU caused intestinal tumors in mice and pancreatic tumors in rats. Liver tumors were caused in mouse, hamster, and rat offspring after gestational exposure to X rays, urethane, and ENU. In addition, transplacental ENU exposure led to a higher incidence of multiple tumor types in the offspring than when the ENU was given to juvenile patas monkeys (Rice et al. 1989).

Along with gestation, other critical periods of offspring development have been examined for carcinogenic toxicant effects. Several carefully conducted studies have examined the carcinogenicity of the industrial chemical ethylene thiourea (ETU) (Chhabra et al. 1992) and the antiseizure medication diphenylhydantoin (DPH) (Chhabra et al. 1993a) in adult rats and mice with or without perinatal exposure. The objective of these studies was to determine whether incorporating exposure during the perinatal period in addition to adult lifetime exposure would enhance the sensitivity of the testing protocol to identify carcinogenic potential. In both series of studies, the maternal rodents were exposed before breeding and throughout gestation, lactation, and weaning, and the resulting offspring were exposed in the diet until 8 weeks of age (perinatal); the rodents were exposed for 2 years starting at 8 weeks of age (adult). In a third group, the rodent exposure was both perinatal and 2 years of adult exposure. Whereas perinatal exposure to DPH alone was only weakly hepatocarcinogenic in female mice, combined perinatal and adult exposure led to a significant increase in liver tumors in male mice compared with adult exposure alone. No effect of perinatal exposure alone was seen in rats for that compound. In the studies involving ETU, perinatal exposure alone was not carcinogenic in rats or mice. However, whereas adult exposure caused thyroid tumors in both rats and mice, the combined exposure regime—i.e., perinatal and adult—further increased the number of ETU-induced thyroid tumors in rats. In neither of these studies did the use of the perinatal exposure paradigm alter the tumor latency period. However, perinatal exposure combined with adult exposure did lead to an enhanced number of tumors in mice in one case and rats in another, suggesting that not only critical periods of development but length of exposure are important for some compounds.

An exposure paradigm similar to that of ETU and DPH was used to examine the carcinogenicity of polybrominated biphenyls (PBBs), a commercial mixture used as a flame retardant (Chhabra et al. 1993b). In this instance, perinatal exposure alone did lead to an increase in liver tumors in both male rats and male and female mice, as did adult exposure alone in all four treatment groups. Combined perinatal and adult exposure enhanced the incidence of liver tumors relative to adult-only exposure. These results confirm and extend an earlier study demonstrating a weak carcinogenic effect in rats exposed to PBBs during gestation (Groce and Kimbrough 1984).

Finally, it must be considered that although several studies may demonstrate that a particular life stage is important for adult end points, the applicability to human disease may differ by species because of dose sensitivity, tissue-specific differences, or differences in the hormonal milieu. For example, there is consistent evidence that *in utero* and immediate *post utero* exposure of rats to the artificial sweetener saccharin results in an increase in bladder tumors when compared with adult exposure only (Taylor et al. 1980), and that in second-generation saccharin-exposed male rats there is a dose-dependent increase in the incidence of bladder tumors (Schoenig et al. 1985). However, in review of studies of adult rats, mice, hamsters, and humans exposed to saccharin, it has been concluded that this compound is safe for humans (Council on Scientific Affairs, 1985; IARC 1999); rats are the only species that possess the urinary pH necessary to cause crystallization of the compound in the bladder, leading to its carcinogenic effects in that species.

Thus, as seen from all the preceding information, industrial chemicals, drugs, and radiation have been associated with an elevated incidence of neoplasms in both experimental animals and in humans after early life-stage exposures. These studies also suggest that fetal susceptibility (e.g., lack of metabolism, protective barriers not formed), sensitive populations (strain differences), and critical periods of target-organ development are key elements in the response to environmental carcinogens.

Endocrine Disruption

There is increasing concern for adverse health outcomes after developmental exposure to environmental compounds that perturb the endocrine system (Birnbaum 1994a). Endocrine-disrupting compounds (EDCs) can be defined as exogenous agents that change endocrine function and cause adverse effects at the level of the organism, its progeny, and/or subpopulations of organisms (U.S. EPA 1997). Gestational and perinatal exposures to EDCs may have long-term effects on the endocrine system that can influence tumor development later in life. The discovery of the transplacental carcinogenic effect of DES, a synthetic estrogen originally used to prevent miscarriage, lends support to this hypothesis. The fact that a synthetic estrogen could cause cancer in offspring should not be surprising, given that elevated levels of natural estrogens during gestation have been associated with an increase in breast cancer in the children of such women

(Weiss et al. 1997). Further, a recent study of 3,613 men suggests that prenatal DES exposure is associated with testicular cancer (Strohsnitter et al. 2001). The increase in testicular cancer may be related to early life-stage exposure to environmental estrogens and/or antiandrogens (Skakkebaek et al. 2001). However, at this time there is little epidemiologic data for other hormonally mediated cancers after developmental exposure.

Endocrine Disruptors and Rodent Tumors

In contrast to the limited epidemiologic data, the amount of data demonstrating the importance of EDC effects after prenatal exposure and their effects in adult tumor formation is increasing in laboratory animal studies. The animal data are particularly strong after exposure to environmental estrogens. McLachlan et al. (1980) demonstrated that prenatal DES exposure in mice resulted in neoplasias of the uterus and genital tract abnormalities. A high incidence of uterine adenocarcinomas (Newbold et al. 1990) and male reproductive tract tumors could also be produced after acute neonatal exposure to DES. Studies suggest that the carcinogenic effects of DES may be transmitted to succeeding generations (Newbold et al 2000; Walker and Kurth 1995).

Genistein is a naturally occurring phytoestrogen found in most soy products. Although early reports suggested beneficial effects of such isoflavenoids, other studies correlate high levels of phytoestrogen intake with adverse health effects. Hilakivi-Clarke et al. (1999) showed an increase in carcinogen-induced mammary cancer in female rat offspring after maternal genistein injection, suggesting that an elevated estrogenic environment *in utero* could increase subsequent breast cancer risk. Exposure of late-gestation dams to genistein resulted in enhanced sensitivity of the pups to DMBA-induced mammary tumors at sexual maturity. However, this result was not repeated in another laboratory (Lamartiniere et al. 2002) when genistein was administered orally. Whether this is solely a function of dose or compound availability remains to be determined. Newbold et al. (2001) injected neonatal mice with genistein (increased bioavailability) and observed an incidence of adenocarcinomas of the uterus similar to that previously observed for DES. Although there are differences in the route of exposure between some of the rodent genistein studies (injected) and human consumption (oral), there were similar route of administration discrepancies in DES studies comparing rodent (injection) versus human (oral) endometrial cancer. There was agreement between the animal and human studies in the latter case (reviewed above). With this said, there is a dramatic lack of epidemiologic studies evaluating the effect of maternal (fetal) or infant soy consumption and correlation with breast, uterine, or testicular cancer.

Tamoxifen, regarded as an estrogen antagonist in the adult breast but an agonist during development and in the adult liver, also acted in an estrogenic manner in the developing mammary gland. Exposure of the rat to tamoxifen during late gestation resulted in enhanced sensitivity to DMBA-induced breast cancer in the offspring at maturity (Hilakivi-Clarke et al. 2000). This was similar to the response previously observed with injected genistein.

The PBBs, possessing multiple endocrine-modulatory effects including estrogenic perturbation, are associated with an increase in liver tumors in both rats and mice after prenatal exposure (Chhabra et al. 1993b). They belong to a class of toxic chemicals known as polyhalogenated aromatic hydrocarbons (PHAHs), which includes the polyhalogenated dibenzo-*p*-dioxins, dibenzofurans, biphenyls, and naphthalenes. The members can be chlorinated, brominated, or have mixed chloro-bromo substitution. The PHAHs provide the best examples of persistent EDCs in the environmental setting. Multiple examples of endocrine disruption caused by members of this class have been observed in wildlife (fish, birds, and mammals) and domestic animals (e.g., cows, sheep, horses, chickens), as well as in a host of laboratory animals, with representatives from all vertebrate classes including nonhuman primates (Birnbaum 2000). For example, the disruption of reproduction in trout of the Great Lakes in the United States has been associated with exposure to dioxin (U.S. EPA 1993), and the lack of reproduction of wild mink has been attributed to environmentally relevant dietary levels of polychlorinated biphenyls (PCBs) (Brunstrom et al. 2001).

Dioxin-Induced Changes in Health

The term "dioxins" is used for members of the PHAHs that are structurally related (have similar halogen substitution patterns), are persistent and bioaccumulative, and have a common spectrum of biologic responses mediated via binding to a specific high-affinity cellular protein, the aryl hydrocarbon (Ah) receptor (Van den Berg et al. 1998). The prototype chemical for this class is dioxin, or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). Often called the most toxic man-made compound, dioxin can cause a host of effects. Although essentially all vertebrates are sensitive to its effects, not every effect may occur in all species. In addition, many of the responses are developmental stage- or sex-dependent.

Dioxins are developmental and reproductive toxicants and cause immunotoxicity, dermal and hepatic toxicity, a plethora of endocrine effects, and cancer (Birnbaum 1994b). Dioxins cause effects at all levels of biologic organization from the molecular level to biochemical changes affecting metabolism, cellular communication, and tissue and organ function. Many of the effects of dioxins in tissues and organs are associated with alterations in proliferation, differentiation, and homeostasis. Dioxins perturb many hormone systems, and depending on the system, the effects may be at the level of the receptor, with either an increase or decrease in the receptor number (e.g., exposure-induced decrease in glucocorticoid receptor number in the liver vs. an increase in that receptor in the developing palate) (Abbott et al. 1994; Lin et al. 1991). TCDD can alter the metabolism of hormones, e.g., estrogen, via the induction of biotransformation enzymes (Safe et al. 1998). Dioxins can also alter the transport of hormones by affecting protein binding in the blood, e.g., thyroid hormone transport by transthyretin (Birnbaum 1994b). In addition to effects on classical hormonal systems, dioxins perturb multiple growth factors, their receptors, and their downstream cell-signaling cascades. These growth factors include EGF, TGFα, EGF receptor, TGFβ, vitamin A, and multiple cytokines, specific interferons and interleukins, as well as signal-transduction components such as those involving tyrosine phosphorylation and calcium mobilization (Birnbaum and Tuomisto 2000).

In addition to its actions as an endocrine disruptor, TCDD is a known human carcinogen (IARC 1997; U.S. Department of Health and Human Services 2000; U.S. EPA 2001), causing an increase in total cancers. Several occupational studies suggest that dioxin exposure is associated with an increase in lung cancer (reviewed in above references). Additional cancers reported after dioxin exposure include hematologic cancers and soft-tissue sarcomas. At least 18 studies have been published on animal cancer in rats, mice, hamsters, and fish, demonstrating cancer-positive outcomes in both sexes and at multiple sites. Experimental studies have also demonstrated that dioxins are potent tumor promoters, enhancing both the incidence and multiplicity of tumors at multiple sites after initiation with a direct-acting mutagen.

The breast is a key tumor-forming organ for which there is apparently conflicting information. The human data are not yet clear, although a recent Seveso, Italy, cohort study demonstrates a significant increase in breast cancer in association with elevated serum levels of dioxin (Warner et al. 2002). An earlier ecologic epidemiologic study on the Seveso population suggested there might be a nonsignificant decrease in the incidence of breast cancer (Bertazzi 1993). In only one of the 17 lifetime rodent studies (Kociba et al. 1978) was there a decrease in the incidence of mammary tumors. However, in certain *in vivo* situations dioxin can be antiestrogenic

(Safe et al. 1998), and in specific cell culture systems dioxin can downregulate the estrogen receptor, resulting in antiestrogenic properties (Gierthy et al. 1993). In contrast, a number of TCDD responses, such as liver tumors in rats and endometriosis in rodents and monkeys, require the presence of estrogen (Birnbaum and Cummings 2002; Lucier et al. 1991).

Prenatal Endocrine Disruption and Mammary Tumors

The model used to demonstrate the prenatal effects of tamoxifen and genistein on the DMBA induction of breast tumors in the adult was used by Brown et al. (1998) with TCDD. These investigators exposed rats prenatally to a single oral dose of TCDD, cross-fostered the animals to eliminate lactation exposure, and treated the pups at sexual maturity with DMBA. The prenatal TCDD treatment led to twice as many mammary tumors several months later. This raises the question of how prenatal TCDD increases the sensitivity of the pups to DMBA-induced mammary cancer. Brown and co-workers did not observe any effects on estrus cyclicity or any evidence of clear estrogenicity of the dioxin treatment, but did note an increased number of terminal end bud structures at the time of DMBA exposure, suggesting that TCDD delayed gland maturation.

The Ah receptor, which is required for dioxin's effects, is present during organogenesis in most tissues (Abbott et al. 1995). It continues to be expressed in the mammary gland of the pubescent rodent and is localized to the mammary ducts and developing lobules (Hushka et al. 1998). In addition, these authors demonstrated that mice in which the Ah receptor has been eliminated display decreased mammary gland size and suppressed lobule development, suggesting a critical role of the Ah receptor in normal and TCDD-exposed mammary gland development.

The rodent mammary gland begins formation as an epithelial bud late in fetal development. The epithelial cells then grow out over the underlying mesenchymal fat pad, leading to an extensive development of ducts with terminal end buds. Following puberty, these terminal end buds differentiate into lobules [reviewed by Fenton et al. (2002)]. Although it has been known for several years that prenatal TCDD can decrease pup body weight, delay vaginal opening, and lead to the induction of persistent vaginal thread (Flaws et al. 1997; Gray and Ostby 1995; Gray et al. 1997), the work of Brown et al. (1998) was the first to demonstrate an effect of TCDD on mammary tissue such as an increase in number of terminal end buds at sexual maturity, a time when in controls they have mostly differentiated into lobules (the precursor structures needed for future lactation).

Recent work (Fenton et al. 2002) has demonstrated that *in utero* exposure to TCDD (1 µg/kg) in rats causes changes in the mammary gland of the offspring, including fewer primary ducts, stunted epithelial progression into the fat pad, fewer than half the number of terminal buds than in control glands, decreased lateral ductal branching, delayed lobule formation, and the presence of terminal end buds at a time when control glands have few. It is important to note that terminal end buds are sensitive to tumor initiation, because they are undergoing rapid proliferation and differentiation, and it is widely believed that the stage of development is key in susceptibility to carcinogenesis (Russo and Russo 1978). Although Brown et al. (1998) were unable to detect any morphologic difference in the prenatally treated mammary glands at weaning, the more recent studies detected alterations in mammary gland development as early as postnatal day (PND) 4 (Fenton et al. 2002). Prenatal dioxin exposure, in the latter studies, also led to the absence of full maturation of the gland, with no evidence that the mammary gland ever recovered. Because Brown et al. (1998) cross-fostered their rats after birth, the possibility existed that lactational exposure was necessary for the very early effects of dioxin noted in Fenton et al. (2002). Therefore, the critical window for these dioxin effects was identified. Pregnant rats were exposed to TCDD (1 µg/kg) at the end of organogenesis [gestation day (GD) 15], just before birth (GD 20), and several days after birth, and the offspring mammary gland was examined at weaning. The lack of epithelial outgrowth and decreased branching was evident only when the exposure occurred in late organogenesis (GD 15), and not after. The GD-15, but not GD-20, dose administration was during a critical phase of mammary bud outgrowth and led to fetal reprogramming of the gland development.

There are functional consequences of dioxin exposure, in addition to the enhanced sensitivity to carcinogens shown by Brown et al. (1998), to this alteration in mammary gland structure. Data reported by Rogan et al. (1987) suggested there was a negative correlation between duration of lactation and measured serum PCB levels in a group of North Carolina women. In a two-generational study (Fenton et al. 2000), sexually mature, prenatally exposed female Long Evans rat offspring were bred with control males and evaluated for second-generation effects. To assess the lactational ability of first-generation rodent pups exposed to TCDD *in utero*, a lactational challenge was conducted on dams with equal-sized litters on PND 10. Dams were removed from the pups for a set amount of time, pups were weighed, dams were returned for a 30-min suckling period, again they were removed, and pups were reweighed. The control pups gained nearly four times more weight during the short suckling period than did the dioxin-exposed offspring (Fenton et al. 2000). Further examination revealed that not only did the dioxin second-generation pups have disrupted mammary gland development, but also exhibited an increase in pituitary weight. This preliminary finding is interesting, given that dioxin exposure of the original dams caused a decrease in serum prolactin levels. A decrease in maternal prolactin is potentially responsible for the decrease in lactation, but may also be associated with an increase in circulating estrogen levels. Could elevated maternal estrogen be associated with the enhanced sensitivity to carcinogens? Is the lack of milk-borne prolactin important in development of the mammary gland in TCDD-exposed offspring? Regardless of the actual mechanisms, prenatal TCDD appears to alter the proliferation and differentiation of the mammary gland, prolonging the window of sensitivity to carcinogenesis. Whether the same mechanism is involved in the increase in number and incidence of methylnitrosourea (MNU)-initiated tumors after neonatal TCDD exposure on PND 18 (Desaulniers et al. 2001) remains to be determined. These investigators also demonstrated that a mixture of other organochlorines, including PCBs, could increase the number of MNU-initiated tumors when the mixture was given neonatally.

Atrazine—Another Developmental Toxicant of the Mammary Gland

The chlorotriazine herbicides currently represent the most heavily used of all agricultural pesticides. The most common of these chemicals is atrazine (ATR), to which nearly 60% of the population of the United States is currently exposed daily. ATR is used to control annual grasses and broadleaf weeds in fields of corn, sorghum, and sugar cane, and in orchards, among other uses [reviewed by Das et al. (2001)]. Regulatory concerns exist because dietary ATR caused mammary cancer in adult Sprague-Dawley rats. However, this effect appeared because of the induction of a premature reproductive senescence, during which time the elevated serum estrogen levels in the rat acted as a tumor promoter to enhance replication in estrogen-sensitive neoplastic cells of spontaneously initiated tumors [reviewed by Eldridge et al. (1998)]. Cooper et al. (2000) have shown that ATR disrupts the hypothalamic control of pituitary–ovarian function, including a decrease in circulating prolactin and luteinizing hormone levels.

Ongoing work (Greiner et al. 2000) demonstrated that ATR exposure altered pubertal mammary gland development. In fact, oral ATR administration inhibited differentiation of the mammary epithelium in

ovariectomized rats exposed to pharmacologic levels of estradiol and progesterone. Further studies (Fenton S. Unpublished data) in which pregnant rats were exposed to ATR during late gestation (postorganogenesis) suggest a delay in normal epithelial progression into the fat pad, detected as early as PND 4. This delay in gland development was evident after sexual maturity and led to an increased presence of terminal end buds, structures sensitive to carcinogen in TCDD studies (Brown et al. 1998). Therefore, ATR exposure *in utero* not only delays early mammary gland development in female offspring, but may also confer an extended window of sensitivity to potential carcinogens after sexual maturity. Delayed mammary gland development of the offspring may be triggered by the altered hormonal milieu of the exposed dam in addition to any direct effect of the exposure on the offspring.

A comparison of mammary gland whole mounts from the same postnatal days in TCDD- and ATR-exposed female rat offspring is shown with control tissue in Figure 1. Are there parallels between the *in utero* effects of TCDD and ATR on mammary gland development, as well as on the susceptibility of the offspring to any carcinogen-initiated tumorigenesis? Both compounds alter the endocrine status of the dam (Cooper et al. 2000; Fenton et al. 2002). These EDCs alter migration of the epithelium into the neonatal mammary fat pad and delay or persistently alter mammary gland development, and in doing so they lengthen the window of sensitivity to potential carcinogens. Whether the actual mechanism underlying these alterations in structure and susceptibility are the same remains to be determined, as is any similarity of these mechanisms in humans.

More EDCs that Potentiate Changes in Mammary Development

Recently, Markey et al. (2001) demonstrated that *in utero* exposure of mice to bisphenol A leads to alterations in mammary gland development in the mouse. Not only did they see a decrease in ductal migration of the epithelium into the stromal compartment, but they also noted a permanent increase in the number of terminal ducts and terminal end buds. Such changes are associated with enhanced susceptibility to carcinogenesis in both rodents and adults and are reminiscent of the changes reported above for both TCDD (Fenton et al. 2000, 2002) and ATR (Fenton S. Unpublished data). Preliminary reports from Waalkes et al. (In Press) have demonstrated that gestational exposure of mice to arsenic results in tumors in multiple tissues in the offspring followed for 104 weeks. In addition to lung and liver tumors, target sites for arsenic carcinogenesis in humans, the pattern of tumors resemble that seen after developmental exposures to estrogens: liver and adrenal tumors in males; ovarian, oviduct, and uterine tumors in females. Whether this apparent estrogenic effect of arsenic is due to the up-regulation of estrogen receptor alpha, as observed in mouse liver after chronic exposure (Chen et al. 2002), remains to be determined.

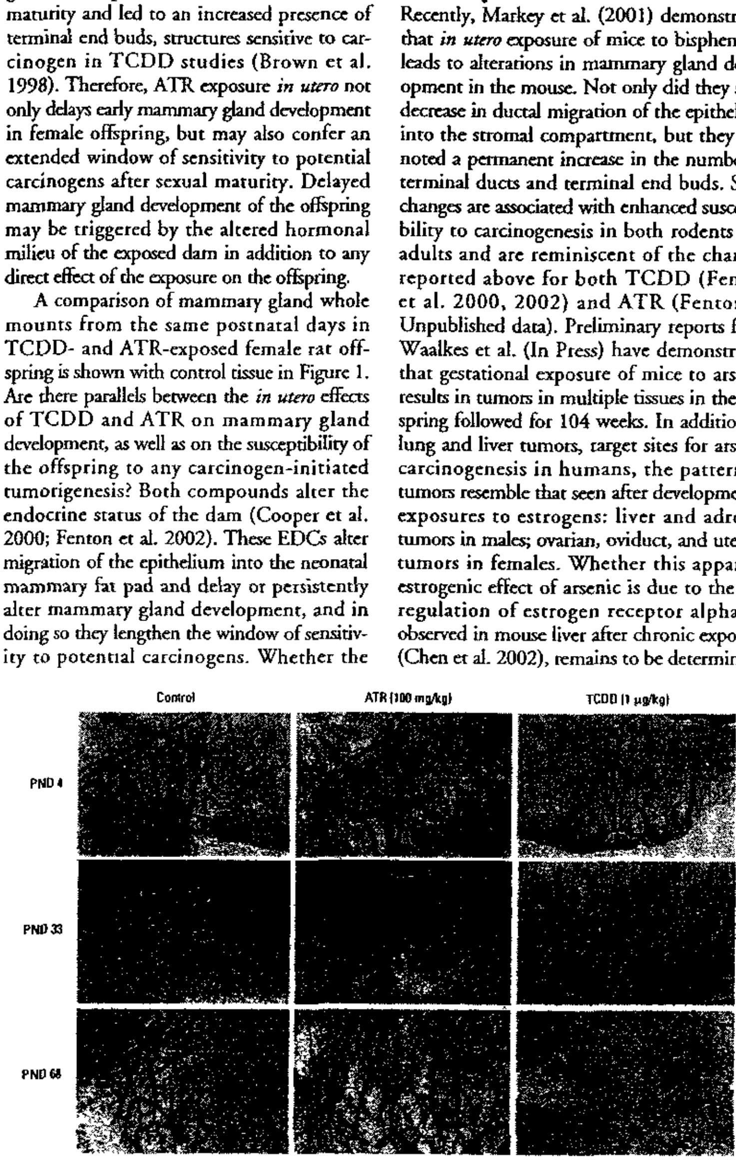

Figure 1. Mammary gland whole mounts from Long Evans rats exposed during late gestation to ATR, 100 mg/kg/day, or TCDD, 1 µg/kg/day. Mammary tissue was compared in female offspring on PNDs 4, 33, and 68 In both treated groups, early disruption of mammary development was evident (PND 4), distinguished by fewer primary ducts from the nipple, lack of lateral branching and budding, and fewer terminal structures. Epithelium in toxicant-exposed glands progressed more slowly through the mammary fat pad and had delayed differentiation (PND 33). Delays in mammary development continued in ATR- and TCDD-exposed animals and were also present at PND 68. The effect of TCDD was more dramatic at later stages, when compared with ATR, presumably because of the compound's persistent nature.

Human Impact?

All of these studies have demonstrated that prenatal exposure to EDCs can alter the hormonal milieu, reproductive tissue development, and susceptibility to potential carcinogen exposure in the adult. These compounds are not genotoxic, yet can have significant adverse health outcomes. Interestingly, dioxin exposure was recently shown to be associated with a delay in breast development in girls (Den Hond et al. 2002). We must ask these questions: Are the appropriate, sensitive animal strains being used to test for endocrinologically based diseases, such as breast cancer? Are many of the adult rodents whose brain and endocrine function are fully developed relatively insensitive when exposed to EDCs as adults? There have been epidemiologic studies investigating the association of environmental chemicals, including both organochlorines, such as PCBs and ATR, with breast cancer incidence (Sasco 2001). These particular studies have measured the levels of exposure of these chemicals in adult women who develop breast cancer. Could we be trying to correlate exposure and effect at the wrong time? If it is prenatal or early life-stage exposure that is critical to disease susceptibility, why are we measuring environmental chemicals in people once they have developed breast cancer? The critical exposure window may have occurred much earlier.

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